Journal of Stress Physiology & Biochemistry, Vol. 20, No. 2, 2024, pp. 26-33 ISSN 1997-0838

ORIGINAL ARTICLE

OPEN ACCESS

The Influence of Sulfuric Acid Salts and Radiation on the Activity of the Enzyme Carbonic Anhydrase in Cotton Ontogenesis

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Received January 21, 2024

Article deals γ-radiation doses of 5, 10, 50, 100, 200, 300 Gy, sulfate (Na_2SO_4, $ZnSO_4$) concentrations of 5, 10, 50, 100, 200, 300 mM of cotton *Gossypium hirsutum L*. The dynamics of changes in the activity of carbonic anhydrase (CA, carbonate hydrolyase, EC 4.2.1.1) enzyme were studied in the ontogeny of the Ganja-182 cultivar of the species *Gossypium hirsutum* L. in type of true leaf emergence (LP), budding (BP), flowering (FP) and opening of seed boll phase (OBP). It was determined that chloride and sulfate salts have different effects on CA activity. Thus, CA activity increases at 200 Gy dose of γ-radiation, 100 mM of NaCl, and 200 mM of $ZnSO_4$. It seems that the increase in CA activity in the medium containing $ZnSO_4$ is related to the increase in the demand for CO_2 under stress. The obtained results show that radiation and sulfate salts have a more regulatory effect on salt adaptation than other salts in cotton plant.

Key words: Gossypium hirsutum L., γ-radiation, salt stress, CA-activity, adaptation

It is shown in the literature that ~25% of the earth's surface in modern times (Hasan *et al.,* 2011), and ~20% of arable land is saline. According to statistics, the world population is expected to reach 10 billion by 2025 (Cafi, 2003). One of the preventive measures taken to prevent the impending global threat on our planet can be the selection of durable and high-yielding cultivated plant varieties that can grow in extreme conditions. From this point of view, the study of physiological-biochemical mechanisms of adaptation to abiotic stress in plants has great scientific and practical importance. According to the results of Banzet *et al.,* (1998), after γ-irradiation, 15 and 17 kDa polypeptides with a protective function are synthesized in the cytosol of the tomato plant, and 22 kDa molecular weight polypeptides are synthesized in the mitochondria (Banzet *et al.,* 1998). Optimum dose of γ-irradiation of seeds accelerates the development of cultivated plants and shortens the ripening period, as a result, grain, potato, etc. increases the productivity of plants by approximately 5-20% (Calabrese, 2011; Jan *et al.,* 2012; Kozmin *et al.,* 2015). From this point of view, importance should be given to the application of radiation as an important biological method to increase the productivity of plants in agriculture (Sanzharova *et al.,* 2016).

The growth, development, productivity and sustainability of plants also depend on the lability of physiological-biochemical processes and the amount of accumulated reserve substances (He, Hou, 2014). In this way, the membrane and cytosol of the plant cell, which has a role in creating photosynthetic productivity (Guliev *et al.,* 2003), chloroplast and mitochondria (Yu *et al.,* 2004), the CA enzyme localized in different subcellular fractions has different physiological functions (Wu *et al.,* 2006). Various isoforms of CA are involved in photosynthesis, respiration, ion transport, carbon-fixing mechanism (CCM) in higher plants (Xiao *et al.,* 2015; Fan *et al.,* 2015; Price *et al.,* 2008) role is to regulate the diffusion and exchange of CO_2 between the cell and the environment (Sun *et al.,* 2014).

The main goal of this study is to compare the influence of different types of radiation, chloride and sulfate salts on the dynamics of CA enzyme activity in the ontogeny of the cotton plant.

MATERIALS AND METHODS

For the goal research purposes, were taken Ganja-182 species of cotton *Gossypium hirsutum* L. Cotton seeds were irradiated with doses of 5, 10, 50, 100, 200 and 300 Gr using Co^{60} as a source of radiation in the RUXUND 20,000-irradiation device at the "Isotope Sources of Radiation" scientific experiment department of the Institute of Radiation Problems of ANAS. Irradiated and non-irradiated seeds were disinfected in 3% H_2O_2 for 15 min before sowing, washed 2-3 times with distilled water after disinfection and transferred to a thermostat in Petri dishes. Seedlings obtained from non-irradiated seeds were planted in vegetation containers with 5, 10, 50, 100, 200, and 300 mM solutions of Na_2SO_4, and $ZnSO_4$ separately, at a temperature regime of 25-28ºC, a photoperiod of 14 hours, humidity of 60-70%, and light intensity. It is placed in an artificial climate chamber with 15-20 klux.

Gas exchange parameters - photosynthesis rate (P_n, μmol CO_2 $m^{-2}s^{-1}$), concentration of CO_2 in intercellular areas (C_i, μmol CO_2 mol^{-1}), permeability of stomatal cells (g_s, mol CO_2 $m^{-2}s^{-1}$) and transpiration rate (T_r, mmol CO_2 $m^{-2}s^{-1}$) was measured in the leaf using an infrared photosynthetic analyzer (LI 6400 XT Postable Photosynthesis System; LI-COR 6400 Biosciences, USA).

To get the **enzyme extract**, the leaves were washed, dried with filter paper and homogenized at +4oC by adding 5 ml of homogenization solution to each gram of leaf. (5 mM DTT, 1 mM EDTA, 20 mM $MgCl_2$, 0.5% PVP, and 0.5% Triton X-100 containing 100 mM , pH 7.8-8.0 by adding Tris-HCl). To obtain the enzyme extract, the leaves were washed, dried with filter paper, and 5 ml of homogenization solution (5 mM DTT, 1 mM EDTA, 20 mM $MgCl_2$, 0.5% PVP, and 0.5% Triton X-100 containing 100 mM , pH 7.8-8.0 by adding Tris-HCl) and homogenized at +4ºC temperature. Obtained homogenates were filtered through double capron, first 5 min at 1000g and then 15 min at 5000g to get rid of the nucleus and non-degradable plant remains. The sediment was discarded, and the supernatant liquid was

used for further work.

CA activity was determined electrometrically according to Wilbur Anderson based on the separation of H^+ ions formed by the reaction $CO_2 + H_2O \rightarrow H^+ + HCO_3^-$ (Wilbur, Anderson, 1948). Released H^+-ions were recorded with pH-meter ЭВ-74 and XY-RECORDER endim 620.02 potentiometer. CA activity was calculated according to Risky (Rickli *et al.,* 1964).

The **total amount of proteins** was determined spectrophotometrically (Ultrospec 3300 pro, Amersham) at a wavelength of 750 nm according to Lowry's method (Lowry *et al.,* 195).

Statistical analyses. The values shown in the tables are mathematical averages and reflect the standard deviation. During the analysis of the results of the study, the average mathematical errors and deviations (M±m) were taken into account. Differences were considered significant when the accuracy probability was R≤0.05. The obtained results were processed using "Microsoft Office Excel 2010" computer programs.

RESULTS AND DISCUSSION

As we know, CA enzyme localized in leaves and roots of higher plants is strongly dependent on the level of gas exchange parameters. Therefore, we have studied the activity of CA enzyme in cotton leaves in relation to gas exchange parameters. The results obtained during the study of gas exchange parameters are given in table 1 (table 1). In our previous experiments, we determined the stimulating effect of 50 Gy dose of radiation and 50 mM concentration of salts on the development of biological processes in cotton plants. Therefore, when studying the parameters of gas exchange in cotton leaves, the optimal radiation dose was 50 g, and the optimal concentration of salts was 50 mM. Therefore, when studying the parameters of gas exchange in cotton leaves, who took 50 g as the optimal radiation dose and 50 mM as the optimal concentration of salts. Later, the effects of radiation and 2 types of salt on the parameters of gas exchange in the leaves of the cultivated cotton plant at this dose and concentration were studied comparatively. As can be seen from the table, the increase of Na_2SO_4 concentration up to 50 mM causes different changes as a result of the effect on P_n, g_s, CO_2 concentration (C_i) and T_r in the intercellular spaces. In green leaves, T_r and P_n are strongly regulated by g_s. Accordingly, as C_i increases, P_n decreases. Compared to C in the GP, BP, and FP of P_n, it increased by 54.5, 37.4, and 15%, respectively.

As can be seen from the first table, the amount of P_n and T_r in 50 mM $ZnSO_4$ concentration decreases, while the amount of C_i increases. Given the significant inverse correlation between the values of these three quantities, based on CO_2 exchange, we studied the activity of CA enzyme in the BP, FP and OBP phases of cotton plant ontogenesis, taking into account its important role in the formation of photosynthesis intensity and photosynthetic productivity. In the presence of $ZnSO_4$, compared to C in the LP, BP and FP phases, Pn is 40.7, 51.9 and 17.1%, respectively, and C_i is 23.9, 14.8 and 19.4 %, respectively increased (table 1).

In order to compare the changes in gas exchange parameters under the influence of 50 mM $ZnSO_4$ in the plant development phases, the values in the BP and FP phases were separately compared with the indicators in the LP phase. It was determined that the values of gs, T_r and P_n decreased over time depending on the phases. Among the gas parameters, only a small increase in the amount of C_i was observed. C_i increased by 5.6% in the BP phase compared to the GP phase, and by 13.6% in the FP phase compared to the BP phase. In the conditions where the amount of C_i increased, the amount of P_n in the boll phase decreased by 14.8% compared to the Leaf phase, and in the flowering phase by 39.9% compared to the green phase. The fact that gas parameters are also low in C in the last stages of vegetation can be explained by leaf senescence. Along with the parallel reduction of P_n and T_r due to the effect of salt, regeneration of CO_2 along with photochemical reactions is also inhibited (Fig. 1).

As we know, one of the most important functions of CA localized in plant cells is to carry out the transport and assimilation of inorganic carbon (CO_2) generated in plant metabolism and fixed from the atmosphere to the carboxylation centers of photosynthesis. If we pay attention to the table, we can see that the CA activity is much higher in the variants with $ZnSO_4$ than in the

variants with other salts. In the second place are Na_2SO_4, and in the following places there are options with $ZnSO_4$ salt. We attribute the fact that the activity of CA enzyme under the influence of $ZnSO_4$ is higher than the activity of CA under the influence of other salts with the presence of the Zn atom, which plays the role of a coenzyme, in the active center of the CA enzyme. 50 mM $ZnSO_4$ stimulates plant growth and development, productivity by increasing CA activity up to 50% compared to 50 mM Na_2SO_4. Such functions give the CA enzyme an adaptive property (Fig. 1).

CA transports CO_2 and HCO_3^- as an inorganic carbon carrier. CA participates in various biological processes by regulating the CO_2/HCO_3^- ratio near the active center (Fan *et al.,* 2015). Back in 2004, Moskvin *et al.* (2004) proved that PS II contains a 33 kDa molecular weight protein with CA activity in its oxygen-releasing complex.

As can be seen from table 1, CA loses its activity faster under the influence of chloride salts. This process is completely opposite to sulfate salts. Although the change of CA activity during ontogenesis in the presence of Na_2SO_4 occurs similar to $ZnSO_4$, the level of CA activity in the presence of $ZnSO_4$ is parallel higher than that of Na_2SO_4. Under the influence of this salt, the biological productivity of the plant increases significantly compared to others. The obtained results may be related to the physiological functions performed by the CA molecule in the spatial structure, organs and tissues.

Figure 1 shows the results obtained regarding the influence of chloride and sulfate salts on the dynamics of CA enzyme activity in cotton plant leaves. As can be seen from the figure, the CA activity in C in the roots of 10, 20 and 30-day-old plants gradually increases, while in the leaves of the plant, on the contrary, it decreases. On the 10th, 20th and 30th days of plant development, 50 and 100 mM NaCl causes a decrease in CA activity in roots and a parallel increase in leaves (Fig. 1).

During the effect of $FeCl_3$ on the CA activity in the roots and leaves of cotton plants, it was determined that in contrast to NaCl, the CA activity in the roots and leaves decreases similarly under the influence of $FeCl_3$ at a concentration of 50 - 100 mM in 10, 20 and 30-day-old plants. These show that the increase in salt concentration in roots and leaves at 50-100 mM concentrations of NaCl and $FeCl_3$ further accelerates the inhibition of CA activity. Such an effect of chloride salts on CA activity in the roots and leaves of cotton plant can be explained by the decrease in the activity of H^+-pumps as a result of the increase in the amount and concentration of salts in the rhizosphere, and in connection with this, the weakening of mineral nutrition, and the disruption of osmotic processes as a result of the accumulation of salts in cells. CA activity in roots and leaves of 10-day-old cotton plants decreases under the influence of $ZnSO_4$ at concentrations of 50 and 100 mM from sulfate salts, while CA activity in 20-day-old plants increases rapidly and reaches the highest level in all variants. In contrast, although a weak reduction (10-15%) occurred in 30-day-old cotton plants, CA activity in all variants was approximately 40-50% higher than in 10- and 20-day-old plants (Fig. 1).

As can be seen from the table, the effect of sulfate salts on CA activity in the roots and leaves of cotton plants is different from the effect of chloride salts. Thus, CA activity in C in 10-day-old plants gradually decreases under the influence of Na_2SO_4, CA activity takes the highest value under the influence of Na_2SO_4 at a concentration of 50 mM, and this indicator decreases over time. Under the influence of Na_2SO_4 at a concentration of 100 mM, the CA activity in the roots and leaves of 20-day-old plants increases, and on the contrary, it gradually decreases in the roots and leaves of 30-day-old plants.

The obtained results show that unlike chloride salts, sulfate salts, including $ZnSO_4$, have a more regulatory effect on the physiological-biochemical processes carried out in the leaf and root cells of the cotton plant, depending on the stages of plant ontogenesis and salt concentration. We attribute this effect of $ZnSO_4$ to the presence of a Zn atom in the active center of the CA enzyme, which performs the coenzyme function. It is shown in the literature that the CA enzyme, which is localized in different subcellular fractions of cells and tissues of C_3 plants and performs various physiological and biochemical functions, has an oligomeric structure and each of its monomers contains 1 g-eqv zinc atom (Idayatov, 1990).

In order to confirm the role of Zn atom in the activation of CA enzyme localized in the leaf cells of cotton plant, we studied the inhibitory effect of orthophenanthroline (OPT), which forms a complex compound with heavy metal salts, on the enzyme activity. OPT precipitates by forming a complex combination with Zn atoms in the active center of CA and in the environment. As a result, the activity of the CA enzyme is completely inhibited, while P_n is simultaneously reduced to a minimum. For this purpose, in order to study the inhibitory effect of OPT on the activity of the CA enzyme obtained from the leaves of the bamboo plant cultivated in the environment containing 50, 100 and 150 mM $ZnSO_4$, the activity of the enzyme was determined at concentrations of 1-5 mM of OPT and the obtained results were comparatively analyzed (Fig. 2).

As can be seen from the picture, OPT at a concentration of $3•10^{-3}$M has completely inhibited CA by combining Zn atoms in the active center of CA enzyme. This process depends on the ambient temperature, pH and concentration of Zn^{2+} ions. Our experiments show that as the concentration of $ZnSO_4$ salt increases to 50-100 mM, CA activity increases accordingly (Fig. 2).

The obtained results can be considered as one of the components of CCM, which arose as a way of adaptation to stress in evolution. CA activity, including flowering phase, increases as the age of the plant increases. After this phase, after remaining relatively unchanged for a certain period, it gradually weakens until the end of vegetation.

Table 1: Effects of radiation, chlorine and sulfate salts on gas exchange parameters during the active development phases of cotton plant ontogeny

Parameters	Control	Sulfate salts, 50 mM		Radiation, 50 Qy
		Na_2SO_4	$ZnSO_4$	
True leaf formation phase (TL)				
P_n	33,2±3,1	45,8±4,83	46,7±4,22	49,9±1,09
C_i	201±18,1	243±20,4	249±14,7	264±21,7
G_s	4,9 ±0,91	6,1±1,00	6,9±0,77	4,01±1,01
T_r	2,2 ±0,61	5,9±1,12	6,55±0,83	6,02±1,43
Budding phase (BP)				
P_n	26,2±3,1	35,9±2,58	39,8±2,3	49,8±1,5
C_i	229±19,7	254±19,8	263±14,7	282±24,8
G_s	4,1± 0,98	5,5±1,23	4,6±1,12	4,91±0,93
T_r	1,8±0,42	4,18±0,94	4,21±0,99	6,14±0,95
Flowering phase (FP)				
P_n	24,0±3,95	28,1±3,88	28,1±2,1	54,1±7,63
C_i	237±20,3	266±19,81	283±19,51	269±20,71
G_s	4,6±1,11	4,8±1,89	4,21±0,42	5,55±1,22
T_r	1,0±0,09	1,12±0,45	1,83±0,18	2,4±0,44

Note: P_n-photosynthesis rate-μmol $CO_2•m^{-2}•s^{-1}$; C_i - the amount of CO^2 in the intercellular spaces – μmol $CO_2•mol^{-1}$; g_s-permeability of the stomata - mol $H_2O•m^{-2}•s^{-1}$, T_r- intensity of transpiration-mmol $H_2O•m^{-2}•s^{-1}$

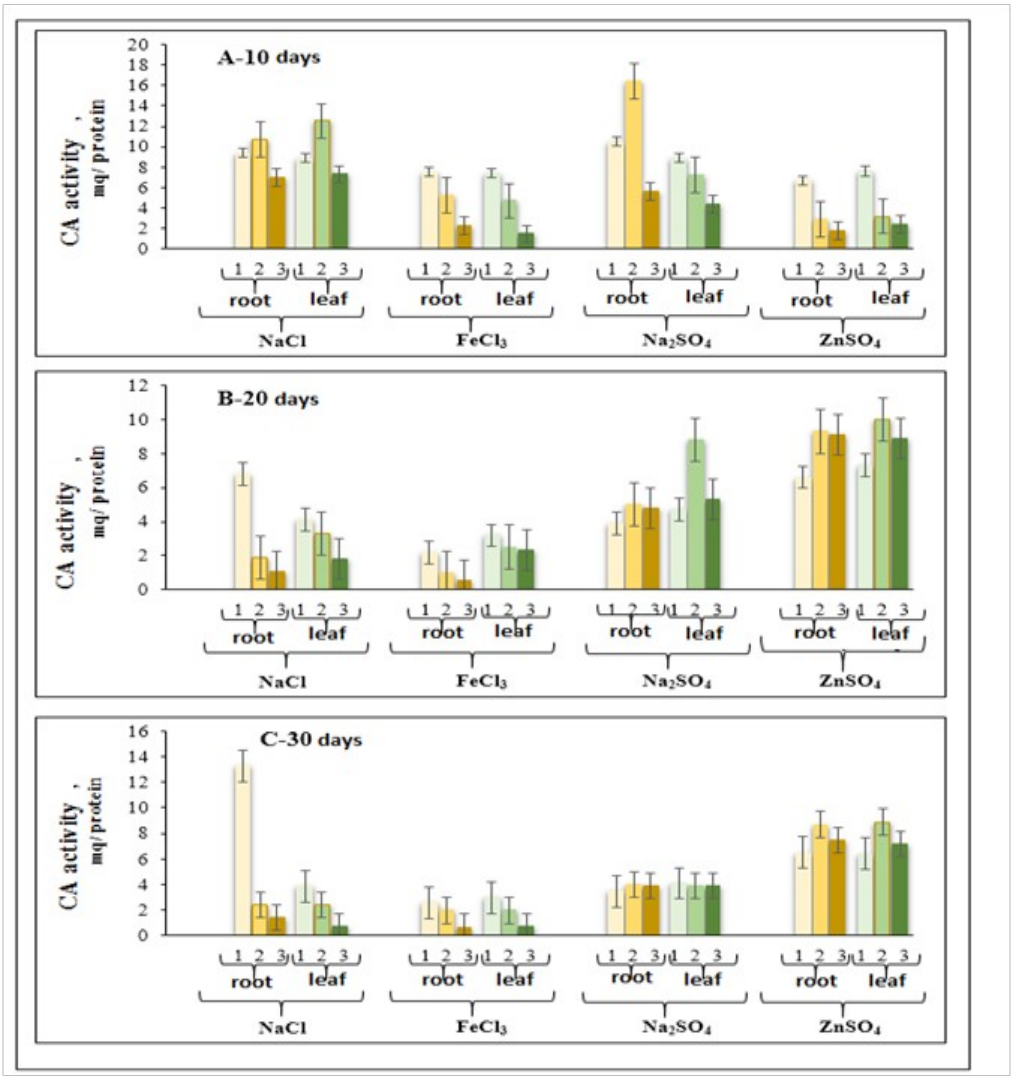

Figure 1. The effect of chloride and sulfate salts on the dynamics of CA enzyme activity in cotton plant leaves. 1-Control; 2-50 mM salt; 3-100 mM salt.

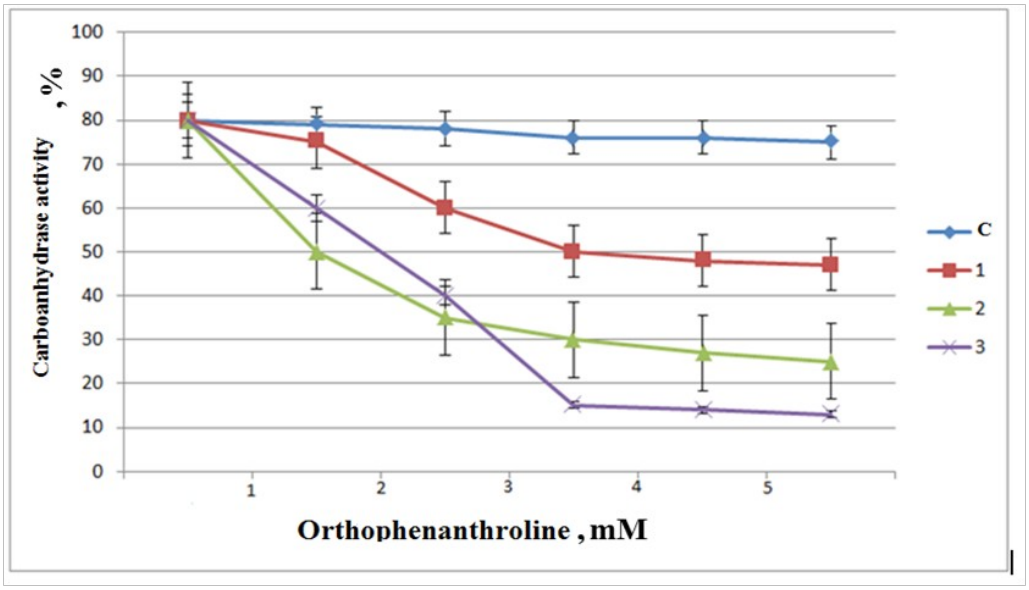

Figure 2. Inhibitory effect of OPT on CA activity in leaves of cotton plants grown in different concentrations of $ZnSO_4$ during the flowering phase (FP) of ontogeny. C-Control, 1-50 mM, 2-100 mM, 3-150 mM $ZnSO_4$

CONCLUSION

The processes occurring in the metabolism of the cotton plant under the influence of radiation and different types of salts: the interrelated changes in the indicators of gas exchange parameters, plant mineral nutrition, and the activity and functional diversity of the CA enzyme isoforms localized in roots and leaves are the physiological and physiological adaptation of plants to stress, can be considered as one of the components of biochemical mechanisms. Sulfate salts have a greater stimulating effect on CA enzyme activity than chloride salts. 50 Gy of radiation and 50 mM $ZnSO_4$ cause an increase in CA activity, further intensification of Pn and a high yield of FP in cotton ontogenesis. Under these conditions, the amount of C_i decreases.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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